Amendments to the Specification:

Please replace the paragraph inserted at page 1 before the first paragraph by way of preliminary amendment filed November 24, 1998, with the following amended paragraph:

This application is a divisional of U.S. Application Serial No. 08/471,494, filed June 6, 1995, now U.S. Patent No. 5,840,853, and a continuation of U.S. Application Serial No. 08/468,249, filed June 6, 1995, now U.S. Patent No. 5,886,148, both of which are divisionals of U.S. Application Serial No. 07/864,475, filed April 6, 1992, now U.S. Patent No. 5,494,806, which ~~was~~ is a continuation-in-part of U.S. Application Serial No. 07/681,702, filed April 5, 1991, and now abandoned.

Replace the paragraph at page 3, line 22 to page 5, line 12, with the following amended paragraph:

The invention features isolated DNA comprising a DNA sequence encoding a cell receptor, preferably a parathyroid hormone receptor, of a vertabrate animal, which receptor has an amino acid sequence with at least 30% (preferably at least 50%, even more preferably at least 60%, and most preferably at least 75%) identity to the amino acid sequence shown in Figs. 3A-E (SEQ ID NO:20) ~~Fig. 3 (SEQ ID NO:3)~~: i.e., when the closest match is made between the two amino acid sequences (using standard methods), at least 30% of the amino acid residues of the former sequence are identical to the amino acid residues of the latter sequence. By "isolated" is meant that the DNA is free of the coding sequences of those genes that, in the naturally-occurring genome of the organism (if any) from which the DNA of the invention is derived, immediately flank the gene encoding the DNA of the invention. The isolated DNA may be single-stranded or double-stranded, and may be genomic DNA, cDNA, recombinant hybrid DNA, or synthetic DNA. It may be identical to a naturally-occurring, cell receptor (e.g. PTH receptor) encoding DNA sequence, or may differ from such sequence by the deletion, addition, or substitution of one or more nucleotides. Single-stranded DNAs of the invention are generally at least 8 nucleotides long, (preferably at least 18 nucleotides long, and more preferably at least

30 nucleotides long) ranging up to full length of the gene or cDNA; they preferably are detectably labelled for use as hybridization probes, and may be antisense. Preferably, the isolated DNA hybridizes under conditions of high stringency to all or part of the DNA sequence show in Figs. 1A-E ~~Fig. 1~~ (SEQ ID NO.: l), Figs. 2A-E ~~Fig. 2~~ (SEQ ID NO.: 2), Fig. 3A-E ~~Fig. 3~~ (SEQ ID NO.: 3), or Figs. 6A-G ~~Fig. 6~~ (SEQ ID NO.: 4). By "high stringency" is meant, for example, conditions such as those described herein below for the isolation of human kidney PTH receptor cDNA (also see Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1989, hereby incorporated by reference). Most preferably, the animal is a mammal (such as an opossum, a rat, or a human), and the DNA sequence encodes substantially all of the amino acid sequence shown in Figs. 1A-E ~~Fig. 1~~ (SEQ ID NO.:l), Figs. 2A-E ~~Fig. 2~~ (SEQ ID NO.:2), Figs. 3A-E ~~Fig. 3~~ (SEQ ID NO.:3) or Figs. 6A-G ~~Fig. 6~~ (SEQ ID NO.:4); or is encoded by the coding sequence of one of the plasmids deposited with the American Type Culture Collection (ATCC) and designated ATCC Accession No. 68570 or 68571. The DNA of the invention may be incorporated into a vector [which may be provided as a purified preparation (e. g., a vector separated from the mixture of vectors which make up a library)] containing a DNA sequence encoding a cell receptor of the invention (e.g. parathyroid hormone receptor) or fragment of the receptor, and a cell or essentially homogenous population of cells (e.g., prokaryotic cells, or eukaryotic cells such as mammalian cells) which contain the vector (or the isolated DNA described above). By "essentially homogenous" is meant that at least 99% of the cells contain the vector of the invention (or the isolated DNA, as the case may be). Preferably, this vector (e.g., R15B) is capable of directing expression of a parathyroid hormone receptor (for example, in a cell transfected or transformed with the vector).

Replace the paragraph at page 8, lines 2-18, with the following amended paragraph:

Figs. 1A-E, inclusive, are ~~Fig. 1~~ is a representation of the nucleic acid (SEQ ID NO.: 1) and amino acid (SEQ ID NO.: 18) sequences for the opossum kidney PTH/PTHrP receptor clone, OK-H. ~~(SEQ. ID NO.: 1)~~

Figs. 2A-E, inclusive, are ~~Fig. 2 is~~ a representation of the nucleic acid (SEQ ID NO.: 2) and amino acid (SEQ ID NO.: 19) sequences for the opossum kidney PTH/PTHrP receptor clone, OK-O. ~~(SEQ ID NO.: 2)~~

Figs. 3A-E, inclusive, are ~~Fig. 3 is~~ a representation of the nucleic acid (SEQ ID NO.: 3) and amino acid (SEQ ID NO.: 20) sequences for the rat bone PTH/PTHrP receptor clone, R15B. ~~(SEQ ID NO.: 3)~~

Fig. 4 is a comparison of the deduced amino acid sequences of R15B (SEQ ID NO:20) (top line) and OK-O (SEQ ID NO:19) (bottom line) encoded by cDNAs from clones OK-O (SEQ ID NO:2) and R15B (SEQ ID NO:3).

Fig. 5 is a comparison of the deduced amino acid sequences of OK-O (SEQ ID NO:19), OK-H (SEQ ID NO:18) and R15B (SEQ ID NO:20), lined up according to sequence homology.

Figs. 6A-G, inclusive, are ~~Fig. 6 is~~ a representation of the nucleic acid (SEQ ID NO.: 4) and amino acid (SEQ ID NO.:21) sequences for the human PTH/PTHrP receptor. ~~(SEQ ID NO.: 4)~~

Replace the paragraph at page 9, lines 9-11, with the following amended paragraph:

Fig. 15A ~~Fig. 15~~ is a graph illustrating accumulation of inositol bisphosphate ~~phosphate~~ metabolism (IP_2) ~~by~~ after NlePTH ~~in~~ stimulation of COS cells transfected with OK-H, OK-O, or R15B.

Fig. 15B is a graph illustrating accumulation of inositol triphosphate (IP_3) after NlePTH stimulation of COS cells transfected with OK-H, OK-O, or R15B.

Replace the paragraph at page 9, lines 15-22, with the following amended paragraph:

Fig. 17A is a graph ~~Fig. 17 are graphs~~ illustrating binding of ^{125}I-labelled PTH(1-34) ~~(A and B) and ^{125}I labelled PTHrP(1-36) (C and D)~~ to COS-7 cells transiently expressing the human kidney ~~(A and C) and the rat bone (B and D)~~ PTH/PTHrP receptor; competing ligands included PTH(1-34)(—□—) ~~(□)~~, PTHrP(1-36) (—O—) ~~(*)~~, PTH(3-34) (—▽—) ~~(■)~~, and PTH(7-34) (—△—) ~~(+)~~.

Data are given as % specific binding and represent the mean±SD of at least three independent experiments.

Fig. 17B is graph illustrating binding of ^{125}I-labelled PTH(1-34) to COS7 cells transiently expressing the rat bone PTH/PTHrP receptor, competing ligands included PTH(1-34) (—□—), PTHrP(1-36) (—O—), PTH(3-34) (—▽—), and PTH(7-34) (—△—). Data are given as % specific binding and represent the mean ± SD of at least three independent experiments.

Fig. 17C is a graph illustrating binding of ^{125}I-labelled PTHrP(1-36) to COS-7 cells transiently expressing with the human kidney PTH/PTHrP receptor; competing ligands included PTH(1-34) (—□—), PTHrP(1-36) (—O—), PTH(3-34) (—▽—), and PTH(7-34) (—△—). Data are given as % specific binding and represent the mean ± SD of at least three independent experiments.

Fig. 17D is a graph illustrating binding of ^{125}I-labelled PTHrP(1-36) to COS7 cells transiently expressing the rat bone PTH/PTHrP receptor competing ligands included PTH(1-34) (—□—), PTHrP(1-36) (—O—), PTH(3-34) (—▽—), and PTH(7-34) (—△—). Data are given as % specific binding and represent the mean ± SD of at least three independent experiments.

Replace the paragraph at page 13, line 14 to page 14, line 3, with the following amended paragraph:

Isolation of cDNA and genomic DNA clones encoding the human PTH/PTHrP receptor: A human kidney oligo dT-primed cDNA library ($1.7x10^6$ independent clones) in lambda GT10 and a genomic library of human placental DNA ($2.5x10^6$ independent clones) in EMBL3 (Sp6/T7) (Clontech, Palo Alto, CA) were screened by the plaque hybridization technique (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed. pp. 108-113, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989) with the ^{32}P-labelled (random primed labelling kit Boehringer Mannheim, Germany) BamHI/NotI 1.8kb restriction enzyme fragment encoding most of the coding sequence of the rat bone PTH/PTHrp receptor Figs. 3A-E) ~~Fig. 3~~. The nitrocellulose filters were incubated at 42°C for 4 hrs in a

prehybridization solution containing 50% formamide, 4x saline sodium citrate (SSC; 1X ~~1~~ SSC: 300 mM NaCl, 30 mM NaCitrate, pH 7.0), 2x Denhardt's solution, 10% Dextran sulphate, 100 μg/ml salmon sperm DNA (final concentration). The hybridizations were carried out in the same solution at 42°C for 13-24h. Filters were washed with 2x SSC/0.1% SDS for 30 minutes at room temperature and then with 1X SSC/0.1% SDS for 30 minutes at 45°C. The films were exposed at -80°C for 18-24h using intensifying screens.

Replace the paragraph at page 22, line 31 to page 23, line 8, with the following amended paragraph:

Two independent clones (OK-H and OK-O), both of which were isolated from the OK cell cDNA library, had lengths of approximately 2 kilobases. The determined nucleotide sequence and predicted amino acid sequence of these clones are shown in Figs. 1A-E ~~Figs. 1~~ (SEQ ID NO.: 1) and Figs. 2A-E ~~2~~ (SEQ ID NO.: 2) respectively. The R15B clone isolated from the ROS cell cDNA library had a length of approximately 4 kilobases. The determined nucleotide sequence and predicted amino acid sequence of the rat bone PTH/PTHrP receptor is depicted in Figs. 3A-E ~~Fig. 3~~ (SEQ ID NO.: 3).

Replace the paragraph at page 23, line 22-30, with the following amended paragraph:

Fig. 4 demonstrates the high degree of homology between the amino acid sequences encoded by the cDNAs from OK-O and ROS 15B. There is an overall 87% homology and a 77.8% amino acid identity between these two receptors. This high level of identity over long stretches of amino ~~amnio~~ acids demonstrates that the amino acid sequence of the PTH receptor is evolutionarily conserved to a high degree. This allows the data from both OK-O and R15B to be extrapolated to other species, including human.

Replace the paragraph at page 24, lines 8-34, with the following amended paragraph:

Some G-protein-coupled receptors are encoded by intronless genes (Kobilka et al., Nature 329:75, 1987); Kobilka et al., J. Biol. Chem. 262:7321, 1987; Heckert et al., Mol.

Endocrinol. 6:70, 1992; Kobilka et al., Science 238:650, 1987; Bonner et al., Science 237:527, 1987; Sunahara et al., Nature 347:80, 1990). To isolate a human PTH/PTHrP receptor cDNA, both a human cDNA library and a human genomic library were screened with a probe (BamHI/NotI) representing most of the coding region of the rat bone PTH/PTHrP receptor Figs. 3A-E) ~~Fig. 3~~. Screening the human kidney cDNA library led to the isolation of the clone HK-1 Figs. 6A-G [SEQ ID NO.:4] ~~(Fig. 6) [SEQ. ID NO.:6]~~. Since one of the two EcoRI cloning sites of lambda GT10 proved to be eliminated as a result of the library construction, the HindIII/EcoRI phage fragment containing the cDNA insert and -250 bp of the 37 kb (left) lambda arm was subcloned into the corresponding restriction sites in pcDNAI. DNA sequencing revealed that the cloned cDNA contained -1000 bp of the 3' coding region and -200 bp of the 3' non-coding region including an A-rich 3' end. The coding region 5' to the XhoI site was subsequently used to re-screen the library and led to the isolation of the clone HK-2 which, after subcloning into pcDNAI, proved to contain -1400 bp of the coding region. For the third screening of the library, the PvuII/PstI fragment of HK-2 was used; the isolated clone HK-3 proved to be identical to HK-2.

Replace the paragraphs at page 25, line 17 through page 26, line 31, with the following amended paragraphs:

To isolate the remaining -450 nucleotides of the coding region, poly (A) + RNA from human kidney was reverse transcribed after priming with H12 (Fig. 7). After single strand synthesis, two independent PCRs were performed using two different forward primers: i) a degenerate primer RK-1 based on the 5' coding end of the two previously cloned PTH/PTHrP receptors, OK-O and R15B; and ii) primer RK-2 based on the 5' non-coding region of HPG1. H-26 was used as the reverse primer for both reactions. Southern blot and restriction map analyses confirmed the expected size of the amplified DNA encoding the human PTH/PTHrP receptor. The blunt-ended PCR products encoding the 5' end of the human PTH/PTHrP were cloned into pcDNAI using the dephosphorylated EcoRV sites. Sequence analysis of each PCR clone confirmed their 5' nucleotide difference due to the difference in forward primer sequence,

but revealed otherwise identical sequences. Nucleotide sequencing of both strands of the human PTH/PTHrP receptor cDNA revealed an open reading frame encoding a 593-amino acid protein (Figs. 6A-G, SEQ ID NO.: 4) ~~(Fig. 6, SEQ ID NO.:4)~~.

The full-length human kidney PTH/PTHrP receptor cDNA, HKrk, was constructed using the BamHI/PvuII fragment of PCR clone #2 and HK-2. Using the full-length cDNA encoding the human PTH/PTHrP receptor, Northern blot analysis of total RNA (-10 μg/lane) from human kidney and SaOS-2 cells revealed one major hybridizing DNA species of -2.5 kb (Fig. 19). The XhoI digest of normal human genomic DNA, when probed with the same full-length cDNA (Fig. 20), revealed one major hybridizing species of about 5.5 kb, and two DNA species of 4 and 8 kb which weakly hybridized. These data ~~date~~ suggest that the human PTH/PTHrP receptor is the product of a single gene. This full-length clone was then transiently expressed in COS-7 cells for functional and biological characterization by the methods cited above.

Comparison of the human receptor with the opposum kidney PTH/PTHrP receptor and the rat ~~rate~~ bone PTH/PTHrP receptor[,] revealed 81% and 91% amino acid sequence identity, respectively, and consequently a very similar hydrophobicity plot (Fig. 8). All extracellular cysteines including the two cysteine residues in the presumed signal peptide are conserved, as are all potential, extracellular N-glycosylation sites. A number of the amino acids which were not identical between the human kidney and rat bone PTH/PTHr receptors were found to be conserved between the human and the opposum receptors. These conserved amino acids include an Arg to Leu at 51, an Arg to Trp at 58, an Arg to His at 262, an Asp to His at 358, an Ile to Thr at 422, and a Thr to Leu at 427.

Replace the paragraph at page 27, lines 9-19, with the following amended paragraph:

Fig. 9 demonstrates that COS cells expressing OK-H bind ^{125}I-PTHrP. These data also demonstrate that binding of PTHrP is inhibited when intact PTH (1-34) or PTH analogues ~~anlogues~~ which are shortened at their amino terminus (i.e. the 3-34 and 7-34 analogues, which contain Nle substitutions for methionine at positions 8 and 18 and a tyrosine substitution for phenylalanine at position 34) are used as competitors for binding. Similarly, binding of

^{125}I-NlePTH to COS cells expressing OK-H was inhibited when PTHrP or PTHrP fragments were used as competitors. These data indicate that PTH and PTHrP both bind to the receptor encoded by OK-H.

Replace the paragraph at page 28, line 27 to page 29, line 9, with the following amended paragraph:

Figs. 15A and 15B demonstrate ~~Fig. 15 demonstrates~~ that COS cells expressing R15B or OK-O increase their rate of phosphatidyl inositol hydrolysis, as evidenced by the rapid increase in inositol trisphosphate (IP_3) (Fig. 15B) and inositol bisphosphate (IP_2) (Fig. 15A) accumulation after stimulation of the cells with NlePTH or PTHrP. Conversely, COS cells expressing OK-H did not show any detectable increase in inositol trisphosphate and inositol bisphosphate accumulation after stimulation with NlePTH or PTHrP. These data suggest that the PTH receptor encoded by R15B and OK-O is coupled to phospholipase C, presumably through G_p. Since the only difference between OK-O and OK-H is in the cytoplasmic C-terminal tail, these data strongly suggest that the C-terminus of the PTH receptor encoded by OK-O and R15B is involved in the activation of phospholipase C.

Replace the paragraph at page 30, line 10 to page 32, line 28, with the following amended paragraph:

The biochemical role of the carboxy-terminal tail of the PTH/PTHrP receptor was further investigated by the construction of a carboxy-terminally-truncated rat receptor, R480, by standard PCR technology using R15B as a template and an upstream primer containing a stop codon inserted at position 481. Briefly, the upstream primer was a synthetic oligonucleotide based on nucleotides 1494-1513 of the rat cDNA sequence (see Figs. 3A-E ~~Fig. 3~~; SEQ ID NO.: 3) to which a stop codon and an XbaI cloning site were added. Thirty PCR cycles were carried out, each cycle consisting of 1 min at 92°C for denaturation, 1 min at 60°C for annealing, and 1 min at 72°C for extension. The product was cut with NsiI and XbaI and purified by gel electrophoresis. R15B was sequentially digested with XbaI and NsiI, and the purified PCR

product was then ligated into the XbaI-NsiI cut R15B vector. The resulting plasmid, R480, was amplified in bacteria and sequenced.

Replace the paragraph at page, line 14 to page 32, line 4, with the following amended paragraph:

Analysis of COS-7 cells transfected with the cloned human PTH/PTHrP receptor demonstrated that radiolabelled PTH (1-34) and PTHrP (1-36) (~200,000 cpm) bound to the expressed receptors with similar efficiency (specific binding: 10.1±3.7% and 7.6±6.0%, respectively) (Figs. 17A and 17C) to that observed for COS-7 cells expressing R15B (specific binding: 8.1+3.5% and 7.1+4.1%, respectively) (Figs. 17B and 17D). The expressed human PTH/PTHrP receptors bound PTH (1-34) with 2-fold higher apparent Kd than did the rat bone PTH/PTHrP receptor: ~5 nM versus ~10 nM (Figs. 17A-17D) ~~(Fig. 17)~~. However, despite their high degree of amino acid homology, the two receptors showed significant differences in affinity for PTH (3-34) and PTH (7-34). PTHrP (1-36) displayed a 2- to 4-fold lower affinity for the human PTH/PTHrP receptor than for the rat receptor (~35 nM for HKrk versus ~10 nM for R15B) which appeared more pronounced when PTHrP (1-36) was used as radioligand. The affinities for PTH (3-34) and PTH (7-34) were 7- and 35-fold higher with the expressed HKrK than with R15B (~7 nM versus ~45 nM for PTH (3-34), respectively; ~60 nM versus ~2000 nM for PTH (7-34), respectively). In COS-7 cells expressing either receptor, both PTH (1-34)and PTHrP (1-36) stimulated the increase in intracellular free calcium and cAMP accumulation to the same extent (Fig. 18).

Replace the paragraph at page 32, lines 6 to 28, with the following amended paragraph:

The amino acid sequence of the human PTH/PTHrP receptor displays a very high degree of conservation compared to the bone PTH/PTHrP receptor from rat, a eutherian mammal, while its sequence identity with the PTH/PTHrP receptor of ~~with~~ the opossum, a marsupial mammal, is less marked. Like the opossum kidney and the rat bone receptor, the human kidney receptor induces an increase in both intracellular ~~intra-cellular~~ cAMP and intracellular free calcium when

challenged with either PTH or PTHrP. Despite the high degree of homology between the human PTH/PTHrP receptor and the opossum and rat homologs, the transiently expressed human receptor has some functional characteristics that are distinct from those of the rat bone receptor. These include a slightly higher affinity for PTH (l-34)and a significantly descreased affinity for PTHrP (l-36). Higher affinities were observed for PTH (3-34) and in particular for PTH (7-34), the affinity of which for the human receptor was about 35-fold higher in comparison to the rat bone receptor. These findings may have significant implications for the future development of PTH/PTHrP analoques, since they predict that species-specific tissues would be the appropriate tissues for testing the potency of antagonists (and agonists) *in vitro*.

Replace the paragraph at page 33, line 28 to page 34, line 6, with the following amended paragraph:

Several structural characteristics which can be deduced from the predicted amino acid sequence of the rat PTH/PTHrP receptor (Figs. 3A-E; SEQ ID NO: 3) ~~(Fig. 3)~~ indicate that the PTH/PTHrP is a G-protein-linked receptor. The amino terminus shows characteristic features of a signal peptide, including a hydrophobic domain and the presence of three consecutive leucine residues. This amino acid stretch of 20-28 amino acids may serve as a leader sequence, similar to the amino terminus preceding the extracellular domains of other glycoprotein receptors. There is also a cluster of seven hydrophobic segments which represent putative membrane-spanning domains (Fig. 19).

Replace the paragraph at page 34, line 23 through page 35, line 19, with the following amended paragraph:

Recent comparison with the newly characterized secretin and calcitonin receptors (Ishihara et al., EMBO J 10:1635, 1991; Lin et al., Science 254:1022, 1991) has revealed between 30 and 40% identity between these receptors and the PTH/PTHrP receptor. Although the PTH/PTHrP receptor is more than 100 amino acids longer than the calcitonin receptor, there is an ~32% identify between the amino acid sequences of the opossum kidney PTH/PTHrP

receptor (SEQ ID NO ~~NO~~.: 2) and porcine kidney calcitonin receptor (GenBank accession no. M74420). A stretch of 17 out of 18 amino acids in the putative transmembrane domain VII are identical. Also, two out of four N-linked glycoslyation sites and the position of seven out of eight potentially extracellular cysteines are conserved. Major differences between the two receptors appear to lie in their NH_2-terminal and COOH-terminal domains. Comparison of amino acid sequences of the rat secretin receptor (GenBank accession no. X59132) and the human PTH/PTHrP receptor indicates that there is a 43% identity between these two receptors, with a stretch of 21 out of 25 amino acids of the putative transmembrane domain VII being identical. The similarity between the PTH/PTHrP, calcitonin and secretin receptors suggests that they represent a new family of seven transmembrane-spanning G protein-coupled receptors that activate adenylate cyclase. Given the amino acid sequences of these receptors, those skilled in art would be able to compare these sequences for regions of identity which would be useful in the design of nucleic acid probes which could then be used for the identification and isolation of other receptors which would belong to this family.

Replace the paragraph at page 36, lines 18-27, with the following amended paragraph:

Polypeptides according to the invention include the opossum and rat and human parathyroid hormone receptors as shown in Figs. 1A-E, 2A-E, and 6A-G ~~(Figs. 1-3 and 6)~~, respectively, and any other naturally-occurring receptor which can be produced by methods analogous to those used to clone and express these receptors, or by methods utilizing as a probe all or part of one of the sequences described herein. In addition, any analog or fragment of a PTH receptor capable of binding to a parathyroid hormone or a parathyroid hormone-related protein is within the invention.

Delete the Sequence Listing shown on pages 51-62 of the specification and insert the Sequence Listing filed on November 6, 2000 at the end of the application.